AJH 2007; 20:248–254

Intrafamilial Correlations of Carotid Intima–Media Thickness and Flow-Mediated Dilation in a Siberian Population

Andrew Ryabikov, Sofia Malyutina, Maxim Ryabikov, Tatiana Kuznetsova, Jan A. Staessen, and Yuri Nikitin

Background: Intima–media thickening and impaired endothelium-dependent vasodilation are complex phenotypes determined by genetic and environmental factors. Few studies assessed these phenotypes in the same subjects. The goal of our study was to assess the sex-specific intrafamilial aggregation of ultrasonographic carotid intima–media thickness (IMT) and brachial flow-mediated vasodilation (FMD) in a Siberian population.

Methods: We randomly recruited 81 nuclear families of Caucasian ancestry (129 parents and 157 offspring, mean age 52.4 and 26.3 years) in Novosibirsk, Russia. Carotid artery IMT and brachial artery FMD were assessed by ultrasound. Intraclass correlation coefficients were calculated between first-degree relatives and between unrelated spouse pairs for IMT and FMD in age- adjusted, sex-adjusted, and multivariate-adjusted models.

Results: Multivariate-adjusted correlation coefficients in sib–sib pairs were 0.27 ($P = .042$) for IMT and 0.29 ($P = .049$) for FMD with heritabilities ($h^2 = 2r$) of 0.54 and 0.58, respectively. For IMT, the mother–offspring ($r = 0.17$, $P = .051$) and mother–daughter correlations ($r = 0.28$, $P = .025$) were significant, whereas the father–offspring correlation did not differ from zero ($r = 0.11$, $P = .33$). For FMD the father–offspring ($r = 0.24$, $P = .042$) and father–son correlations ($r = 0.40$, $P = .051$) were significant, whereas the mother–offspring correlation was only -0.09 ($P = .39$). The P value for the difference in familial aggregation of FMD between father–offspring and mother–offspring pairs was .018.

Conclusions: Our findings confirm that a substantial proportion of the variability of carotid IMT and brachial FMD is attributable to genetic variation. They also suggest that offspring share more genetic or environmental determinants of FMD with fathers than their mothers. Am J Hypertens 2007;20:248–254 © 2007 American Journal of Hypertension, Ltd.

Key Words: Intima–media thickness, flow-mediated dilation, heritability, population.

Carotid intima–media thickness (IMT)[1,2] and flow-mediated vascular dilation (FMD)[3] reflect the structural and functional properties of the vasculature and predict cardiovascular outcome. The IMT represents a quantitative marker of generalized atherosclerosis.[4] Increased IMT[5–7] and vascular reactivity[7,8] often go hand in hand with a parental history of hypertension, coronary heart disease, premature myocardial infarction, and stroke. These findings suggest involvement of heritable factors in the determination of these vascular phenotypes. Several studies explored the heritability of either IMT or FMD in twins,[9,10] in selected families with a history of coronary heart disease or diabetes,[11,12] or in a general population.[13–16] However, estimates of heritability of IMT were inconsistent.[12–14,17,18] To our knowledge, only one previous study addressed the familial aggregation of FMD[16] and no study investigated the heritability of both IMT and FMD in the same subjects. Also the gender-specific influences on IMT and FMD in offspring remain largely unknown. To answer these research questions we evaluated the sex-specific intrafamilial aggregation of carotid IMT and brachial FMD in

Received June 1, 2006. First decision August 16, 2006. Accepted September 3, 2006.

From the Laboratory of Internal Medicine and Department of Noninvasive Diagnostics, Institute of Internal Medicine, Siberian Branch of the Russian Academy of Medical Science (AR, SM, MR, TK, YN), Novosibirsk, Russia; and Study Coordinating Centre, Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, University of Leuven (TK, JAS), Leuven, Belgium.

The European Project on Genes in Hypertension was supported by the European Union (contract numbers IC15-CT98-0329-EPOGH and QLG1-CT-2000-01137-EURNETGEN). The Russian Foundation for Basic Research (grant No.06-04-49671) supported the collection of the arterial measurements and analysis. AR is recipient of Doctoral Fellowship of the Russian Academy of Medical Sciences.

Address correspondence and reprint requests to Dr. A. Ryabikov, Institute Int Med, SB RAMS, Bogatkova st, 175/1. Novosibirsk, 630089, Russia; e-mail: andrew_ryabikov@mail.ru

0895-7061/07/$32.00
doi:10.1016/j.amjhyper.2006.09.005

a white (Siberian) population, using a random family-based sampling frame.

Methods

Study Population

In the framework of the EPOGH project (European Project On Genes in Hypertension), we randomly selected from the study population in Novosibirsk (Russian Federation) white nucleus families consisting of at least one parent and two siblings. The age range for participation was 18 to 60 years. The response rate was of 68%. We recruited 324 subjects of whom 302 underwent an ultrasonographic measurements of carotid IMT and 272, a measurement of brachial FMD. Of these, 16 subjects were excluded because they were not part of biological family or family whose members had vascular ultrasound. There were no cases eliminated because of insufficient quality of ultrasound examination. After exclusion of unrelated subjects, 286 and 256 were available for the family-based analyses of IMT and FMD, respectively. The Ethics Committee of the Institute of Internal Medicine (Novosibirsk, Russian Federation) approved the study, which was conducted according the principals of the Helsinki declaration. The participants gave informed written consent.

Clinical Measurements

For at least 3 h before being examined, the participants refrained from heavy exercise, smoking, alcohol, or caffeine-containing beverages. Physicians measured blood pressure (BP) and anthropometric characteristics. A questionnaire was given to collect information about each subject's medical history, smoking habit, and intake of medications. From the type and number of alcoholic beverages consumed each day, we calculated alcohol consumption in grams per day. Each participant's office BP was the average of five consecutive readings. Hypertension was defined as BP equal to or in excess of 140 mm Hg systolic or 90 mm Hg diastolic (The JNC 7, 2003), or when the patients were on antihypertensive treatment regardless of their actual BP. Body mass index (BMI) was defined as weight in kilograms divided by the square of height in meters. With use of published tables, the energy spent in physical activity was calculated from the time devoted to sports and physical activity.[19] The serum concentration of total and HDL-cholesterol and triglycerides, and blood glucose were measured after an overnight fasting by automated methods. The LDL-cholesterol was calculated by the Friedewald formula. According to published criteria,[20] diabetes mellitus was defined as a fasting blood glucose level of at least 7.0 mmol/L, or as the use of antidiabetic drugs. The AB0 blood group and rhesus phenotype were verified for inconsistencies in Mendelian segregation.

Measurements of Carotid IMT

One experienced observer (AR) measured carotid IMT using the SIM 7000CFM ultrasound system (ESAOTE S.p.a., Florence, Italy) with 7.5/10-mHz phased-array transducer. He first performed longitudinal and transverse scans along with Doppler blood flow measurements at the right and left carotid arteries to assess the anatomy and to detect the presence of significant atherosclerotic lesions. Next, he obtained from each side electrocardiogram (ECG)-gated longitudinal images of the 10-mm segment of the distal common carotid artery before the bulb. If plaque was present in the target segment, IMT was measured in the plaque-free zone closest to the target site. The carotid images were analyzed offline. Carotid IMT was the mean of three measurements at the far wall. For analysis, we used the mean IMT value either from the right or left artery, whichever was higher.

According to Bland and Altman's approach,[21] we computed the repeatability coefficient as twice the standard deviation between repeat measurements and expressed it as a percentage of the mean of the measurement under study. The intraobserver intersession repeatability coefficient was 2.4% (Pearson's correlation coefficient, $r = 0.927$) and the intraobserver intrasession coefficient was 2.0% ($r = 0.972$).

Measurements of FMD

After the subjects had rested for at least 10 min in the supine position, one observer (MR) obtained ECG-gated B-mode images of the brachial artery at the right mid-upper arm.[22] Next, he inflated an occluding cuff below the elbow for 5 min to at least 50 mm Hg above the subject's systolic BP to induce ischemia of the forearm. We considered that reactive hyperemia occurred within 30 to 60 sec after cuff deflation. The diameter of the brachial artery was measured offline. For analysis, we averaged three measurements at baseline and during hyperemia. We expressed FMD as the percentage increase in brachial diameter during hyperemia compared to baseline.

For the measurements of brachial diameter, the intraobserver intersession repeatability coefficient was 2.8% ($r = 0.988$) and the intraobserver intrasession coefficient was 2.0% ($r = 0.995$).

Statistical Analysis

For statistical analysis, we used the SPSS software package, version 11.0 (SPSS for Windows, SPSS, Chicago, IL). Comparison of means and proportions relied on the standard normal Z test and the χ^2 statistic, respectively. We calculated the arterial measurements correlation coefficients between members of the same family as a measure of concordance (positive correlation) or discordance (negative correlation). Hence, in the context of this article, the terms correlation and concordance are used interchangeably. For adjustment we selected variables, known to influence IMT and FMD.[4,10,13,16] In addition, we tested univariate models and defined explanatory variables,

based on their correlations with IMT or FMD in the entire sample (gender, age, systolic BP, total and HDL-cholesterol, baseline diameter of brachial artery) or in sex- and generation-specific groups (eg, BMI, smoking, diabetes mellitus, antihypertensive treatment, alcohol intake, physical activity). In the following analyses, we adjusted for confounders in a cumulative and stepwise fashion. First, in model 1, we adjusted only for gender and age. Model 2 also included BMI, systolic BP, and total and HDL-cholesterol. In model 3, we added as explanatory variables total and HDL-cholesterol, antihypertensive treatment, and history of diabetes mellitus. Finally, we considered various lifestyle factors, such as smoking, alcohol intake and physical activity (model 4). In model 4, for FMD, we also adjusted the parent–offspring and spouse–spouse correlation for the diameter of brachial artery at baseline. We derived the significance of the intrafamilial correlation coefficients from a *t* test statistic. We compared correlation coefficients using Fisher's Z-transformation. Finally, we calculated simple estimates of heritability (h^2) from the full-adjusted partial correlation coefficients as $h^2 = 2r$, where r is the between-sibling correlation.[13]

Results

General Characteristics of the Parents and Offspring

Our study population included 129 parents and 157 offspring, and 81 sib–sib, 48 spouse–spouse, and 246 parent–offspring pairs. The number of offspring subjects per family in our sample amounted to 3 or 4 in 5% of families, 2 in 81% of families, and 1 in the rest families. Tables 1 and 2 list the general characteristics and the arterial measurements by generation and gender. Mean age of parents and offspring (±SD) was 52.4 ± 5.52 years and 26.3 ± 5.04 years, respectively. Among parents, women had higher BMI, higher prevalence of diabetes mellitus, and were treated with antihypertensive drugs more often than men. Among offspring, women had lower BP, serum triglycerides, and blood glucose level in comparison with men. In both generations, women compared to men, had lower waist-to-hip ratio and higher HDL-cholesterol, reported a lower daily alcohol intake, and were less likely to be a smoker. In parents as well as offspring, carotid IMT and brachial artery diameter at baseline were lower in women than in men. Women of both generations had higher FMD than men.

Intrafamilial Aggregation of Carotid IMT

The intraclass correlation coefficients for carotid IMT are shown in Fig. 1 for sib–sib, parent–offspring, and spouse–spouse pairs. Multivariate-adjusted (model 4) correlations for IMT were 0.27 (95% CI 0.010 to 0.492; $P = .042$) for sib–sib pairs, 0.10 (95% CI −0.035 to 0.225; $P = .15$) for parent–offspring pairs, and −0.01 (95% CI −0.381 to 0.365; $P = .96$) for spouse–spouse pairs. Gender-specific correlations were statistically significant for mother–offspring pairs ($r = 0.17$; $P = .051$) and mother–daughter pairs ($r = 0.28$; $P = .025$) and did not differ from zero for father–offspring, father–son, father–daughter, and mother–son pairs (Table 3). The mother–offspring correlation coefficient was not significantly higher than the father–offspring correlation ($r = 0.17$ *v* $r = 0.11$; $P = .62$). In addition, controlling for fasting glucose level or premature coronary artery disease (CAD) in the multivariate model did not significantly alter the intrafamilial correlations for IMT (data not shown). For carotid IMT, h^2 was 0.54.

Intrafamilial Aggregation of Brachial FMD

The intraclass correlation coefficients for FMD are shown in Fig. 2 for sib–sib, parent–offspring, and spouse–spouse pairs. Multivariate-adjusted (model 4) correlations for FMD were 0.29 (95% CI 0.002 to 0.537; $P = .049$) for sib–sib pairs, 0.10 (95% CI −0.047 to 0.235; $P = .19$) for parent–offspring pairs, and −0.03 (95% CI −0.415 to 0.360; $P = .88$) for spouse–spouse pairs. Gender-specific correlations were significant for father–offspring pairs, 0.24 ($P = .042$) and father–son pairs, 0.40 ($P = .051$) and were not statistically different from zero for mother–offspring, mother–son, mother–daughter, and father–daughter pairs (Table 3). In addition, controlling for menopausal status in mother-related pairs did not alter the results: correlations for FMD in mother–offspring pairs were −0.09 ($P = .396$), in mother–son −0.19 ($P = .363$), and in mother–daughter pairs −0.09 ($P = .539$), and did not differ from zero. The father–offspring correlation coefficient was significantly higher than the mother–offspring correlation ($r = 0.24$ *v* $r = -0.09$; $P = .018$). In the multivariate analysis additional adjustment for fasting glucose or premature CAD did not attenuate the intrafamilial correlations for FMD (data not shown). For FMD, h^2 was 0.58.

To exclude that the difference between the father–offspring and mother–offspring correlation coefficients for FMD was due to a type I error, we created fictional "parent–offspring" pairs, using the random number function implemented in the SPSS software. We repeated this procedure 10 times. In this analysis of unrelated subjects, the fictional "father–offspring" correlation coefficient ranged from −0.02 (95% CI −0.26 to 0.22; $P = .86$) to 0.04 (95% CI −0.20 to 0.28; $P = .73$). The fictional "mother–offspring" correlation coefficients ranged from −0.04 (95% CI −0.22 to 0.15; $P = .69$) to 0.05 (95% CI −0.13 to 0.23; $P = .57$).

Intrafamilial Aggregation of Height and Body Weight

We also assessed the intrafamilial correlations of height and body weight. After adjustment for sex (only if different) and age, we found sib-sib correlation coefficients of 0.53 ($P < .001$) for height and 0.38 for weight ($P < .001$). The adjusted correlation coefficients for height ranged

Table 1. General characteristics of parents and offspring

Characteristics	Fathers	Mothers	*P*	Sons	Daughters	*P*
Total number (*n*)	54	75		70	87	
Age (y)	53.2 (5.66)	51.8 (5.38)	.161	25.8 (4.51)	26.7 (5.42)	.290
Body mass index (kg/m^2)	26.4 (4.14)	29.9 (4.88)	<.001	23.1 (3.00)	22.2 (3.56)	.104
Waist-to-hip ratio	0.90 (0.07)	0.81 (0.06)	<.001	0.83 (0.05)	0.74 (0.05)	<.001
Systolic BP (mm Hg)*	137.3 (22.52)	141.6 (22.19)	.275	121.8 (13.48)	109.2 (12.29)	<.001
Diastolic BP (mm Hg)*	91.9 (13.54)	90.5 (11.22)	.543	80.2 (10.41)	73.3 (9.59)	<.001
Pulse rate (beats/min)	70.9 (7.86)	71.1 (8.73)	.851	71.5 (9.08)	73.8 (10.39)	.151
Total cholesterol (mmol/L)	5.54 (1.19)	5.55 (1.05)	.951	4.46 (1.16)	4.39 (0.91)	.690
LDL-cholesterol (mmol/L)	4.03 (1.06)	3.97 (0.87)	.724	3.04 (1.03)	2.89 (0.83)	.321
HDL-cholesterol (mmol/L)	0.86 (0.31)	0.97 (0.31)	.050	0.96 (0.32)	1.12 (0.31)	.002
Triglycerides (mmol/L)	2.02 (1.57)	1.87 (1.35)	.556	1.35 (0.74)	1.12 (0.45)	.021
Fasting blood glucose (mmol/L)	4.94 (0.49)	5.00 (0.92)	.677	4.76 (0.51)	4.54 (0.50)	.008
Hypertension, *n* (%)	24 (44.4)	43 (57.3)	.148	9 (12.9)	4 (4.6)	.062
Treated with antihypertensive drugs, *n* (%)	8 (14.8)	24 (32.0)	.026	3 (4.3)	2 (2.3)	.481
Diabetes mellitus, *n* (%)	0	5 (6.7%)	—	0	0	—
Premature coronary artery disease, *n* (%)	4 (7.4%)	3 (4.0%)	.389	0	0	—
Current smoking, *n* (%)	24 (44.4)	1 (1.3)	<.001	39 (55.7)	20 (23.0)	<.001
Alcohol intake per day (g)	14.0 (13.34)	2.4 (3.82)	<.001	17.1 (15.81)	4.3 (4.67)	<.001
Total enegy expenditure (kcal)	1.75 (0.81)	1.51 (0.75)	.082	1.98 (0.97)	1.64 (0.67)	.015

Values are arithmetic means (SD) or number of subjects (%).

* Average of five readings in office.

Table 2. Ultrasound vascular measurements in parents and offspring

Characteristics	Fathers	Mothers	*P*	Sons	Daughters	*P*
Total number (*n*)	54	75		70	87	
Structural measures of the common carotid arteries						
IMT, right artery (mm)	0.71 (0.175)	0.60 (0.126)	<.001	0.47 (0.092)	0.44 (0.069)	.009
IMT, left artery (mm)	0.72 (0.165)	0.62 (0.150)	.001	0.48 (0.088)	0.44 (0.080)	.017
IMT, maximal (mm)	0.76 (0.176)	0.64 (0.153)	<.001	0.50 (0.089)	0.46 (0.074)	.011
Functional measures of the brachial artery						
Baseline diameter (mm)	4.68 (0.578)	3.58 (0.410)	<.001	4.06 (0.533)	3.10 (0.376)	<.001
Diameter during reactive hyperemia (mm)	5.00 (0.648)	3.98 (0.397)	<.001	4.39 (0.535)	3.50 (0.365)	<.001
FMD (%)	7.04 (8.16)	12.25 (7.47)	<.001	8.04 (5.18)	13.50 (7.54)	<.001

IMT = intima–media thickness; FMD = flow-mediated dilation.
Values are arithmetic means (SD).

from 0.54 in mother–daughter pairs to 0.68 in father–son pairs ($P < .001$). For body weight, the adjusted parent–offspring correlations varied from 0.23 to 0.37 ($P < .01$). The correlation coefficient for body weight was not statistically different from zero in spouse–spouse pairs ($r = 0.18$; $P = .22$).

Discussion

We found that FMD was significantly concordant among siblings and in father–offspring pairs, but not in mother–offspring pairs or between spouses. Siblings belong to the same generation, and have more genetic material in common than their parents. Accordingly, after accounting for the main confounders, the intraclass correlation between siblings for FMD was 0.29 with a heritability of 0.58. After similar adjustments, no significant correlation was found among spouse pairs. In line with our findings, numerous studies support the idea that genetic variation modulates endothelial function.[23] Moreover, young individuals with the family history of cardiovascular disease (CVD) have diminished endothelial function.[7,24] Among 2883 participants enrolled in the Framingham Heart Study[16] (52.9% women; mean age 61 years), FMD was inversely related to age, systolic BP, BMI, and smoking, whereas it was positively related to female gender and heart rate. The estimated heritability was 0.14. The influence of lifestyle and environmental factors likely increases as years go by. The older age of Framingham participants might therefore explain why estimated heritability was lower in the former than in the current study.

In fully adjusted models, the intrafamilial aggregation of FMD was significantly higher in father–offspring than in mother–offspring pairs. These findings suggest that offspring share more genetic or environmental determinants of FMD with their father than with their mother. The menstrual cycle influences endothelial function and might have weakened the parent–offspring correlations more in mother–daughter than in father–daughter pairs.[22,25] In our sample the accounting, in particular for menopausal status, did not augment correlations for FMD in mother-related pairs. Furthermore, the HDL-cholesterol level is a determinant of FMD and is associated with genetic variation in the Y chromosome over and beyond the effect of testosterone.[26] Although we adjusted for lifestyle factors, we cannot exclude that residual confoundary, for instance, by smoking and drinking habits or by the usual level of physical activity, inflated the father–offspring particularly the father–son, aggregation in FMD.

We confirmed that carotid IMT is inherited. The sib–sib correlation coefficient was 0.27 and heritability 0.54. In the existing literature[11–15,17,18] estimates of intrafamilial aggregation and heritability of IMT show large variation, which may be related to insufficient sample size, the number of relatives within families, ethnicity, differences in lifestyle or environmental factors, or the presence versus absence of pathologic conditions such as hypertension, carotid atherosclerosis, or type 2 diabetes mellitus. For instance, the estimates of the sib–sib correlations varied from approximate 0.15 in the Framingham Heart Study[13] to approximately 0.38 in Stanislas cohort.[14] Heritability

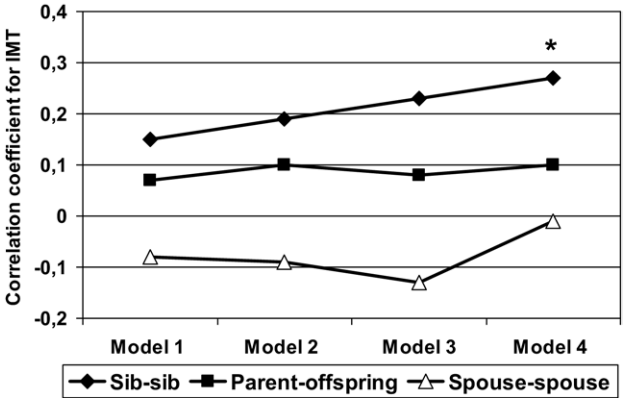

FIG. 1. Intrafamilial correlation coefficients for carotid intima–media thickness (IMT). Model 1 is adjusted for gender and age. The three other models reflect further cumulative adjustments for body mass index and systolic blood pressure (model 2), for total and HDL-cholesterol, history of diabetes mellitus, and antihypertensive treatment (model 3), and in addition for lifestyle factors such as smoking, alcohol intake, and physical activity (model 4). *$P < .05$, significance of the intraclass correlation coefficient.

Table 3. Adjusted intrafamilial correlation coefficients for IMT and FMD*

	IMT			FMD		
Pairs	***n***	***r*(95% CI)**	***P***	***n***	***r*(95% CI)**	***P***
Spouse–spouse	48	−0.01 (−0.381 to 0.365)	.961	48	−0.03 (−0.415 to 0.360)	.876
Sibling–sibling	81	0.27 (0.010 to 0.492)	.042	72	0.29 (0.002 to 0.537)	.049
Parent–offspring	246	0.10 (−0.035 to 0.225)	.149	224	0.10 (−0.047 to 0.235)	.188
Father–offspring	101	0.11 (−0.112 to 0.320)	.330	95	0.24 (0.009 to 0.448)	.042
Father–son	48	0.05 (−0.310 to 0.397)	.789	43	0.40 (−0.024 to 0.704)	.051
Father–daughter	53	0.25 (−0.098 to 0.541)	.157	52	0.22 (−0.143 to 0.535)	.229
Mother–offspring	145	0.17 (−0.001 to 0.340)	.051	129	−0.09 (−0.282 to 0.114)	.394
Mother–son	62	0.15 (−0.152 to 0.435)	.321	52	−0.22 (−0.557 to 0.187)	.290
Mother–daughter	83	0.28 (0.036 to 0.492)	.025	77	−0.12 (−0.345 to 0.159)	.394

IMT = intima–media thickness; FMD = flow-mediated dilation.
* Correlation coefficients are for the fully adjusted model 4.

estimates were about 0.35 in the Framingham Heart Study,[13] 0.41 in families with history of type 2 diabetes mellitus,[12] approximately 0.60 in families with a history of hypertension or carotid atherosclerosis,[27,28] and even about 0.90 in a Mexican cohort of mixed ethnicity.[17]

The present study must be interpreted within the context of its potential limitations. When assessing FMD in population-based sample we could not avoid the administration of CVD medications. However, we have adjusted for antihypertensive treatment. In addition, it was shown that medications not containing nitrates do not significantly influence FMD.[29] Due to substantial prevalence of hypertension in our sample the study results might be primarily relevant to the populations with high risk of hypertension/CVD. We did not measure some of the shared lifestyle factors, which might impact on carotid IMT or brachial FMD, such as the dietary intake of saturated fat or the weather conditions[30] at the time of the FMD measurements. Our sample size was smaller than, for example, in the Framingham Heart Study. On the other hand, we excluded the difference in the familial aggregation of FMD between father–offspring and mother–offspring pairs due to a type I error by creating fictional parent–offspring pairs. In addition, our current estimates of the intrafamilial aggregation of the anthropometric characteristics are in line with published literature and may be considered to represent an external validation of our study results.

In conclusion, our findings confirm that a substantial proportion of the variability of carotid IMT and brachial FMD is attributable to genetic variation. They also suggest that offspring share more genetic or environmental determinants of FMD with fathers than with their mothers.

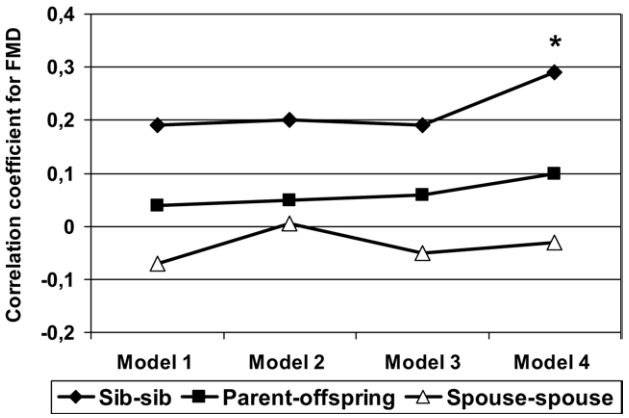

FIG. 2. Intrafamilial correlation coefficients for brachial flow-mediated dilation (FMD). For further explanation, see Fig. 1. In model 4, we also adjusted the parent–offspring and spouse–spouse correlation for the diameter of brachial artery at baseline. *$P < .05$, significance of the intraclass correlation coefficient.

References

1. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK: Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.
2. Bots ML, Hoes AW, Koudstaal PL, Hofman A, Grobbee DE: Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96: 1432–1437.
3. Chan SY, Mancini GBJ, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A: The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. J Am Coll Cardiol 2003;42:1037–1043.
4. Grobbee DE, Bots ML: Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. J Int Med 1994;236:567–573.
5. Zureik M, Touboul P-J, Bonithon-Kopp C, Courbon D, Ruelland I, Ducimetiere P: Differential association of common carotid intima-media thickness and carotid atherosclerotic plaques with parental history of premature death from coronary heart disease. The EVA Study. Arterioscler Thromb Vasc Biol 1999;19:366–371.
6. Wang TJ, Nam BH, D'Agostino RB, Wolf PA, Lloyd-Jones DM, MacRae CA, Wilson PW, Polak JF, O'Donnell CJ: Carotid intima-media thickness is associated with premature parental coronary heart disease: the Framingham Heart Study. Circulation 2003;108: 572–576.
7. Gaeta G, De Michele M, Cuomo S, Guarini P, Foglia M, Bond MG, Trevisan M: Arterial abnormalities in the offspring of patients with premature myocardial infarction. N Engl J Med 2000;343:840–846.

8. Taddei S, Virdis A, Mattei P, Ghiadoni L, Sugano I, Salvetti A: Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. Circulation 1996;94:1298–1303.
9. Jartti L, Rönnemaa T, Kaprio J, Järvisalo MJ, Toikka JO, Marneimi J, Hammar N, Alfredsson L, Saraste M, Hartaila J, Koskenvuo M, Raitakari OT: Population-based twin study of the effect of migration from Finland to Sweden on endothelial function and intima-media thickness. Arterioscler Thromb Vasc Biol 2002;22:832–837.
10. Swan L, Birnie DH, Inglis G, Connell JM, Hillis WS: The determination of carotid intima medial thickness in adults—a population-based twin study. Atherosclerosis 2003;166:137–141.
11. Wang D, Yang H, Quiñones M, Bulnes-Enriquez I, Jimenez X, De La Rosa R, Modilevsky T, Yu K, Li Y, Taylor KD, Hsueh WA, Hodis HN, Rotter JI: A genome-wide scan for carotid artery intima-media thickness. The Mexican-American Coronary Artery Disease Family Study. Stroke 2005;36:540–545.
12. Lange LA, Bowden DW, Langefeld CD, Wagenknecht LE, Carr J, Rich SS, Riley WA, Freedman BI: Heritability of carotid artery intima-media thickness in type 2 diabetes. Stroke 2002;33:1876–1881.
13. Fox CS, Polak JF, Chazaro I, Cupples A, Wolf PA, D'Agostino RA, O'Donnell CJ: Genetic and environmental contributions to atherosclerosis phenotypes in men and women. Heritability of carotid intima-media thickness in the Framingham Heart Study. Stroke 2003;34:397–401.
14. Zannad S, Visvikis S, Gueguen R, Sass C, Chapet O, Herbeth B, Siest G: Genetics strongly determines the wall thickness of the left and right carotid arteries. Hum Genet 1998;103:183–188.
15. Juo S-H, Lin H-F, Rundek T, Sabala E, Boden-Albala B, Park N, Lan M-Yu, Sacco R: Genetic and environmental contributions to carotid intima-media thickness and obesity phenotypes in the Northern Manhattan Family Study. Stroke 2004;35:2243–2247.
16. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF, Lehman BT, Fan Sh, Osypiuk E, Vita JA: Clinical correlates and heritability of flow-mediated dilation in the community. The Framingham Heart Study. Circulation 2004;109:613–619.
17. Duggirala R, Gonzales VC, O'Leary DH, Stern MP, Blangero J: Genetic basis of variation in carotid artery wall thickness. Stroke 1996;27:833–837.
18. North KE, MacCluer JW, Devereux RB, Howard BV, Welty TK, Best LG, Lee ET, Fabsitz RR, Roman MJ: Heritability of carotid artery structure and function: the Strong Heart Family Study. Arterioscler Thromb Vasc Biol 2002;22:1698–1703.
19. McArdle WD, Katch FI, Katch WL: Appendix C: Energy expenditure in household, occupational, recreational, and sport activities. In: Exercise Physiology: Energy, Nutrition, and Human performance. Philadelphia, PA, Lea & Febiger, 1991, pp 804–811.
20. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diab Care 2003;26(Suppl I):S5–S20.
21. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–310.
22. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R: Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–265.
23. Jones LC, Hingorani AD: Genetic regulation of endothelial function. Heart 2005;91:1275–1277.
24. Clarkson P, Celermajer DS, Powe AJ, Donald AE, Henry RMA, Deanfield DE: Endothelium-dependent dilatation is impaired in young healthy subjects with a family history of premature coronary disease. Circulation 1997;96:3378–3383.
25. Taddei S, Virdis A, Ghiadoni L, Mattei P, Sudano I, Bernini G, Pinto S, Salvetti A: Menopause is associated with endothelial dysfunction in women. Hypertens 1996;28:576–582.
26. Charchar F, Tomaszewski M, Lacka B, Zakrzewski J, Zukowska-Szczechowska E, Grzeszczak W, Dominiczak A: Association of the human Y chromosome with cholesterol levels in the general population. Arterioscler Thromb Vasc Biol 2004;24:308–312.
27. Moskau S, Golla A, Grothe C, Boes M, Pohl C, Klockgether T: Heritability of carotid artery atherosclerotic lesions. An ultrasound study in 154 families. Stroke 2005;36:5–8.
28. Xiang AH, Azen SP, Buchanan TA, Raffel LJ, Tan S, Cheng LS, Diaz J, Toscano E, Quinonnes M, Liu CR, Liu CH, Castellani LW, Hsueh WA, Rotter JI, Hodis HN: Heritability of subclinical atherosclerosis in Latino families ascertained through a hypertensive parent. Arterioscler Thromb Vasc Biol 2002;22:843–848.
29. Gokce N, Holbrook M, Hunter LM, Palmisano J, Vigalok E, Keaney JF Jr, Vita JA: Acute effect of vasoactive drug treatment on brachial artery reactivity. J Am Coll Cardiol 2002;40:761–765.
30. Nawrot T, Staessen J, Fagard R, Van Bortel L, Struijker-Boudier H: Endothelial function and outdoor temperature. Eur J Epidemiol 2005;20:407–410.